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Chronic exposure to low doses bisphenol A interferes with pair-bonding and exploration in female Mongolian gerbils

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Abstract

Estrogenic endocrine disruptors, synthetic or naturally occurring substances found in the environment, can interfere with the vertebrate endocrine system and, mimicking estrogens, interact with the neuroendocrine substrates of behavior. Since species vary in their sensitivity to steroids, it is of great interest to widen the range of species included in the researches on neurobehavioral effects of estrogenic endocrine disruptors. We examined socio-sexual and exploratory behavior of Mongolian gerbil females (*Meriones unguiculatus*), a monogamous rodent, in response to chronic exposure to the estrogenic endocrine disruptor bisphenol A. Paired females were daily administered with one of the following treatments: bisphenol A (2 or 20 μg/kg body weight/day); 17α-ethynil estradiol (0.04 μg/kg body weight/day 17αE); oil (vehicle). Females were treated for 3 weeks after pairing. Starting on day of pairing, social interactions within pairs were daily recorded. Three weeks after pairing, females were individually tested in a free exploratory paradigm. Bisphenol A and 17αE affected male–female social interactions by increasing social investigation. Bisphenol A reduced several exploratory parameters, indicating a decreased exploratory propensity of females. These results highlight the sensitivity of adult female gerbils to bisphenol A during the hormonally sensitive period of pair formation, also considering that the bisphenol A doses tested are well below the suggested human tolerable daily intake.

Keywords: Estrogenic endocrine disruptors; Estrogen; Social behavior

1. Introduction

There is now considerable evidence that some substances of anthropic (e.g., pesticides, plastic) or natural (e.g., soy) origin, accumulated in the environment, may have a hormonal-like action and interfere with the functioning of the vertebrate neuroendocrine gonadal axis [19,46]. Many of these endocrine disrupting chemicals act through binding to the estrogen receptors and they have been named estrogenic endocrine disruptors (EEDs). Bisphenol A (BPA) is one of the most common EEDs, which is released by polycarbonate plastics, epoxy resins of food cans, etc., and has been reported to be weakly estrogenic both in vitro and in vivo systems. BPA binds to both α and β estrogen receptors (ERs) with low affinity and transactivates reporter genes in vitro [18,28]. Its developmental and reproductive toxicity at high doses has been reported in rats and mice [14,20,30], whereas the effects of BPA low doses have been controversial [6,31].

Because estrogens are a critical element for determination and regulation of the reproductive and the neuroendocrine systems in vertebrates, exposure to EEDs that interferes with these hormonal signals is likely to impact subsequent reproductive, neuroendocrine and behavioral functions [15,45]. Neurobehavioral alterations induced by EEDs are especially problematic when exposure occurs during critical sensitive developmental stages in the life cycle (e.g., fetal development). Particularly, EEDs can interact with the neuroendocrine substrates of behavior, interfering in the organization and expression of species-specific behaviors essential for survival and reproductive success [7,15,25,44,45]. Among the potential behavioral targets of such interference, social (aggression, group cohesion, courtship, pair bonding, mating and parental care) as well as non-social behaviors (exploration,

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dispersion patterns, orientation, homing, migration) can be included. The type of estrogen-sensitive behaviors depends on the ecology of a species and sex. Reproductive and behavioral effects following maternal exposure to very low doses of BPA have been recently reported in mice [23,36] and rats [10,11,14,26,27].

The majority of the (few) studies examining the effects of EEDs on behavior have been conducted in rats and mice, i.e., polygynous species [14,35,36,40]. However, the expression of socio-sexual behaviors, as well as their hormonal control, may vary in a pattern that is associated with social organization and the mating system of a species. Sex steroid hormones concentrations differ across species [34], potentially accounting for the species-specific sensitivity to hormones [21], which, in turn, may relate to the number and/or distribution of hormone receptors in the central nervous system. This can explain the reported species differences in sensitivity to EEDs.

The purpose of the present study was to investigate the effects of chronic exposure to low doses of BPA during adulthood on social and non-social behaviors in a new animal model, the female Mongolian gerbil (*Meriones unguiculatus*). Mongolian gerbils were chosen in the present study for the following reasons: (1) This species is monogamous, with a social system based on a stable male–female pair and their philopatric young. Reproduction is restricted to the breeding pair, through reproductive suppression in philopatric young and inbreeding avoidance [1]. In the laboratory, gerbils display high levels of affiliation, as cage mates spend most of the time engaging in physical contact [41]. (2) Gerbils, like rats and most other domestic mammals, experience a spontaneous estrus and predictable cyclic variations in hormonal levels [43], thus females are expected to rely at a great extent on gonadal hormones fluctuations. Therefore, although socially monogamous, gerbils have a hormonal cycle similar to that observed in the well characterized polygynous species. (3) The sensitivity of gerbil female behavior to estrogen during the initial phase of the social assessment of a potential mate has been recently demonstrated [39], providing evidences of a critical time window in the adult during which to assess the effects of estrogen-like compounds.

2. Material and methods

2.1. Animals and pairing procedure

Mongolian gerbils were purchased from Charles River (Calco, Como, Italy) at 8–9 weeks of age and housed in our colony in same-sexed groups (four to five animals) in 58 cm × 38 cm × 20 cm Plexiglas cages, with a layer of wood shavings and cotton as nesting material. All animals were kept under a 12-h light/12-h dark cycle (lights on at 06:00) and at a temperature of 21 ± 2 °C. Food (Mil Morini Rodent Chow, Reggio Emilia, Italy) and water were provided ad libitum.

After 4 weeks of acclimation, male–female pairs were set up by simultaneously introducing a male and a female into a clean 58 cm × 38 cm × 20 cm Plexiglas cage, provided with layer of wood shaving and cotton as nesting material.

Procedures reported in the present study were approved by Ministero della Salute, (approval # 44/2001-B) and met the Italian (D.L.vo 116/92) and EEC (86/609/CEE) guidelines for animal care and welfare.

2.2. Treatments

Starting a week prior pairing, gerbil females were daily trained to drink 0.2 ml of corn oil (Sigma Chemical Co.) from a modified syringe (syringe without needle, as previously described [36]). This procedure avoids the stress of chronic treatment. Starting the day of pairing, females were daily administered with one of the following treatments: BPA2 (2 μg/kg body weight/day of BPA, purchased from Sigma Chemical Co., $n = 12$), BPA20 (20 μg/kg body weight/day of BPA, $n = 12$); 17αE (0.04 μg/kg body weight/day of 17α-ethynil estradiol—17αE, purchased from Sigma Chemical Co., $n = 12$), OIL (vehicle, $n = 12$). Due to the oral route of administration, 17αE was chosen as the estrogenic positive control.

From day of pairing to day 21 of cohabitation, each female drunk 0.1 ml/50 g body weight/day of different solutions, which were provided by experimenters 2 h after light onset.

2.3. Pairs' behavior

Social interactions occurring within each pair were daily recorded starting on day of pairing throughout day 21, following an instantaneous sampling method. A one 5-s observation every 2-min per pair schedule (a total of 30 observations per day) was applied. The occurrence of the following behaviors was recorded: agonism (lateral sideways posture and upright posture), social investigation (sniffing head-, mouth-, genital- and ventral-areas of the body), huddling (body contact, independently by a specific location of animals) and sharing nest (nest co-occupancy, performed by animals being in close contact, while performing activities such as nest building, allogrooming, resting or sleeping together). The scores of the behavioral observations were converted to percentages of the possible maximum of each observational period.

Pairs were left undisturbed except for measuring female body weights every three days and for the weekly cleaning of the cages.

2.4. Free exploratory test (FET)

Twenty-one days after pairing, females of all experimental groups were individually tested in a free exploratory test.

The apparatus consisted of two sections: (1) a home-cage (58 cm × 38 cm × 20 cm) in which pairs were housed a week before testing, provided with a layer of wood shavings and